Neural correlates of the emergence of consciousness of thirst

Gary Egan*†, Tim Silk*, Frank Zamarripa‡, John Williams*, Paolo Federico§, Ross Cunnington*, Leonie Carabott*, John Blair-West*¶, Robert Shade¶, Michael McKinley*, Michael Farrell*, Jack Lancaster‡, Graeme Jackson§, Peter Fox‡, and Derek Denton*¶

*The Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Victoria 3010, Australia; ‡Research Imaging Center, University of Texas Health Science Center, San Antonio, TX 78229-3904; ¶Southwest Foundation for Biomedical Research, P.O. Box 760549, San Antonio, TX 78245-0549; and §Brain Research Institute, Austin and Repatriation Medical Centre, West Heidelberg, Victoria 3086, Australia

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Thirst was induced by rapid i.v. infusion of hypertonic saline (0.51 M at 13.4 ml/min). Ten humans were neuroimaged by positron-emission tomography (PET) and four by functional MRI (fMRI). PET images were made 25 min after beginning infusion, when the sensation of thirst began to enter the stream of consciousness. The fMRI images were made when the maximum rate of increase of thirst occurred. The PET results showed regional cerebral blood flow changes similar to those delineated when thirst was maximal. These loci involved the phylogenetically ancient areas of the brain. fMRI showed activation in the anterior wall of the third ventricle, an area that is key in the genesis of thirst but is not an area revealed by PET imaging. Thus, this region plays as major a role in thirst for humans as for animals. Strong activations in the brain with fMRI included the anterior cingulate, parahippocampal gyrus, inferior and middle frontal gyri, insula, and cerebellum. When the subjects drank water to satiation, thirst declined immediately to baseline. A precipitate decline in intensity of activation signal occurred in the anterior cingulate area (Brodmann area 32) putatively related to consciousness of thirst. The intensity of activation in the anterior wall of the third ventricle was essentially unchanged, which is consistent with the fact that a significant time (15–20 min) would be needed before plasma Na concentration changed as a result of water absorption from the gut.

Homeostasis in the vegetative regulatory systems is subserved, *inter alia*, by several specific primary emotions. Functional neuroimaging has delineated the anterior cingulate as a major locus with regard to thirst (1, 2). This region also plays a major role in hunger for air (3–5), hunger for food (6), micturition (7–9), and pain (10, 11), as noted by Liotti *et al.* (3) and Sewards and Sewards (12).

An earlier report (1) of a positron-emission tomography (PET) study of the brain recorded regional cerebral blood flow (rCBF) changes when thirst was maximal, which occurred 40 min after ending a rapid i.v. infusion of 0.51 M NaCl at 11.4 ml/kg per hr. Activations were observed in the anterior and posterior cingulate, parahippocampal and orbital frontal gyri, insula, claustrum, thalamus, and cerebellum. There was also activation in the postcentral gyrus, which disappeared with wetting the mouth (1). Overall, the pattern of activations and deactivations found when thirst was maximal involved phylogenetically ancient areas of the brain. This pattern is consistent with the vegetative systems having emerged early in the transition of vertebrate animals from river and estuarine systems to dry land.

A particular feature of the study was an area of strong activation in Brodmann area (BA) 32/24, at the genu of the corpus callosum. It persisted with wetting the mouth with water, but completely disappeared 3 min after the subjects drank to satiation. We postulated that this area could be a core area associated with the consciousness of thirst (1, 2). In the PET study, it was noteworthy that there was no activation seen in the hypothalamus in the region of the lamina terminalis (the subfornical organ and median preoptic nucleus and the organ vasculosum of the lamina terminalis) (1). This region is crucial for the detection of change in the salt concentration of the blood and the genesis of thirst (13).

It would evidently be of great interest to image the brain at the time when the hypertonic infusion caused thirst to first begin invading the stream of consciousness. In contrast to our earlier report (1), this paper reports the results of imaging after 25 min of infusion, when the subjective sense of thirst had just appeared. Commonality of effects at this point and at the stage of maximum thirst may more firmly indict particular areas as specific to thirst sensation. However, in addition, some areas may alternatively respond to the progressive change of Na concentration or osmolality of the blood as distinct from the subjective sensation of thirst itself.

With the same protocol of hypertonic i.v. infusion, we have used functional magnetic resonance (fMR) imaging to image the changes in the brain when the rate of increase of subjective sensation of thirst was greatest, which was actually during the early stages of consciousness of thirst. Activation of the lamina terminalis region was clearly shown. However, current neuroimaging techniques could not delineate the specific regions within the lamina terminalis. Major activations were seen in the parahippocampal gyrus, anterior cingulate, insula, and frontal gyri. Overall, our aim was to investigate the pattern of brain activity when a hypertonic NaCl infusion first caused thirst to invade the stream of consciousness (PET) or to rapidly change the perception of thirst (fMR). Also we examined the effects of satiation of thirst.

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Methods

The PET and fMR neuroimaging studies were undertaken in two separate subject cohorts.

PET Imaging Protocol. Details of the 10 subjects (male, aged 24–36 years) who participated in this study, the experimental details, and the institutional consent procedures for this experiment have been reported (1, 14, 15). The experimental sequence reported here involved the acquisition of two control (rest) scans and a scan after 25 min of rapid infusion of 0.51 M NaCl at 11.4 ml/kg per hr. The infusion was continued for 50 min. The results of the correlation between the rCBF change of plasma Na concentration (16) and thirst sensation have been reported (1). The latter was based on a rating of 0 for no thirst and a rating of 10 as the most severe thirst experience a subject ever had. The methods used at the Research Imaging Center, University of Texas Health Science Center, including normalization of images to the Talairach and Tournoux atlas (17), have been described in detail (18).

Abbreviations: PET, positron-emission tomography; fMR, functional magnetic resonance; rCBF, regional cerebral blood flow; BOLD, blood oxygenation level-dependent; BA, Brodmann area; EPI, echo planar imaging; SPM, statistical parametric mapping.

†To whom correspondence should be addressed. E-mail: g.egan@hfi.unimelb.edu.au.

fMR Imaging Protocol. Four healthy normal subjects (three male, one female; age range 21–40, mean age 26) gave informed consent in accordance with the Human Research Ethics Committee at the Howard Florey Institute. Subjects were screened to ensure they had no neurological or psychiatric disorders, and each subject had a physical examination and an ECG to exclude any cardiac abnormality. Anatomical and functional scans were acquired with each subject supine with their eyes closed in a dimly lit room and with their head supported in a head fixation device (an evacuated polystyrene ball bag). An i.v. line through which hypertonic saline was to be infused was inserted into each subject's left forearm. Ten minutes of baseline echo planar imaging (EPI) was initially acquired without infusion. Thirst was rated as in the PET study. After 10 min, the hypertonic saline infusion of 0.51 M NaCl commenced; EPI scanning continued, with the scanning being stopped for 10 s every 2.5 min for a thirst score. After stopping the infusion, scanning and thirst score recordings were continued until a steady score was obtained (two identical scores in succession), which involved another 10 min of scanning. The subject was then allowed to drink water to satiation through a tube previously suspended from the head coil. A final 10 min of EPI scanning was then performed.

fMR data were acquired on a 3-T magnetic resonance scanner (General Electric) at the Brain Research Institute in Melbourne. High-resolution anatomical magnetic resonance images were acquired in sagittal orientation for each subject [repetition time (TR) = 11.8 ms; echo time (TE) = 2.4 ms; number of excitations (NEX) = 1; 256 × 256 × 128 matrix; voxel = 0.9 × 0.9 mm^2; slice thickness = 1.4 mm). Transaxially oriented EPI (TR = 3500 ms; TE = 30 ms; flip angle = 60°; 128 × 128 matrix; voxel = 1.98 × 1.98 mm^2; slice thickness = 5.0 mm with no interslice gap; slices = 26) were acquired with more than 900 volumes per subject session (subjects 1, 2, 3, and 4 = 1,153, 918, 906, and 1,286 volumes respectively).

fMR Image Analysis. Statistical analyses were carried out by using statistical parametric mapping (SPM) program SPM99 (Wellcome Department of Cognitive Neurology, London) performed in MATLAB 5.3 (MathsWorks, Natick, MA). Images for each subject were aligned and spatially normalized into Talairach coordinates by using the SPM99 EPI template and smoothed with an 8-mm full width at half maximum smoothing function. The EPI data were modeled as a consecutive series of 1.25-min epochs by using boxcar functions. Pairwise contrasts between adjacent epochs were used to identify areas that showed a mean increase in signal over each 2.5-min interval (i.e., each pair of 1.25-min epochs). A conjunction analysis across four consecutive 2.5-min intervals was then used to identify areas of significant blood oxygenation level-dependent (BOLD) signal activation (19). Conjunction maps for three 10-min periods were calculated for the baseline period, the period of maximum thirst score increase, and the period after drinking. The period of maximum thirst score increase was identified as the 10-min period during the infusion when the subject's thirst score increased by the greatest amount. Voxel-based Z-score statistic maps were calculated for the maximum thirst score increase period, thresholded to a corrected level of $P < 0.0005$ ($Z > 3.54$), and reported where activation cluster sizes were greater than 15 voxels.

Anatomical images were registered to the SPM T1 template image, and the activation maps were overlaid on the spatially normalized anatomical images. Areas were labeled and described in terms of the BAs by using TALAIRACH DAEMON 1.1 client system classification (Research Imaging Center, University of Texas Health Science Center) (17, 18).

Results

PET Behavioral. Plasma Na concentration increased by 3 mmol/liter from the start of the infusion to the time when thirst was beginning to increase after 25 min of i.v. infusion (16). Thirst increased from 0.2 ± 0.2 (mean ± SEM) initially to 1.4 ± 0.5 at the midpoint of the i.v. infusion when a PET scan was recorded. The mean thirst score subsequently increased to 5.3 ± 0.9 at 43 ± 2 min after the infusion finished.

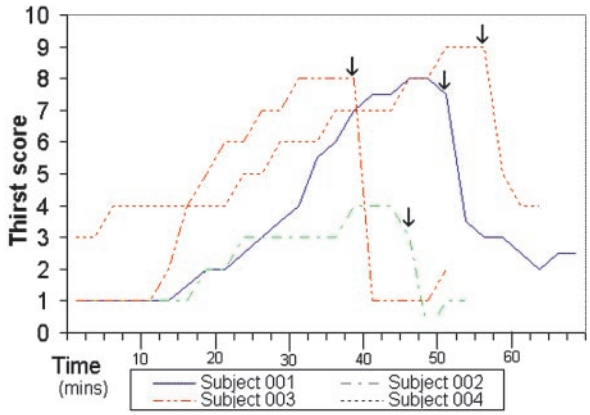

Fig. 1. The subjective thirst score ratings for each subject in the fMR experimental cohort. For each subject, infusion was commenced after 10 min of baseline EPI imaging and was ceased 25–42 min later. Subjects then drank water (indicated by ↓) to satiation from 2.5 to 10 min thereafter, and their thirst ratings were recorded for an additional 10 min.

fMR Behavioral. Three of the four subjects commenced with a baseline thirst score rating of 0, and the fourth subject commenced with a rating of 3. The mean thirst score rating increase across the four subjects was 6.1 ± 2.6 (Fig. 1). Three had a maximum thirst score rating of either 8 or 9. One subject reported a maximum rating of only 4. All subjects' thirst score ratings plateaued after the infusion was ceased and then decreased precipitously to close to their initial baseline level immediately after the subjects drank water.

The average volume of water consumed by the four subjects was 640 ml (range = 350–1,100 ml). The 10-min period in which the rate of thirst score increase was identified as maximal was 15–25 min, 20–30 min, 2.5–12.5 min, and 12.5–22.5 min for subjects 1, 2, 3, and 4, respectively. The brain activity observed during this period is reported in Table 2. Furthermore, for the second subject, who reported a thirst score increase of only 3 units, there was an additional 10-min period of equivalent thirst score increase (7.5–17.5 min).

PET Imaging Results. The early stage of development of thirst caused significant activations and deactivations, which are recorded in Table 1 and Fig. 2. Activations included the left and right culmen of the cerebellum, the left declive, left lingual, and right pyramis. The cingulate areas were the left anterior cingulate (BA 24), right cingulate gyrus (BA 31), left posterior cingulate region (BA 29), left insula (BA 13), and right inferior frontal gyrus (BA 47). There were also activations bilaterally in the parahippocampal gyri (BA 28/30/36), precentral gyrus (BA 4), left postcentral gyrus (BA 1/2/3), right middle occipital gyrus (BA 37), right cuneus (BA 18), and left fusiform gyrus (BA 37).

Deactivations occurred throughout the brain, particularly in the basal ganglia and midbrain. The most significant were in the right nodule of the cerebellum and the left cerebellar tonsil; the caudate was also extensively deactivated bilaterally, including both the right caudate tail and left head of the caudate. The pons was also strongly deactivated. The right cingulate gyrus was deactivated posteriorly (BA 31) and anteriorly in two regions (see Table 1 for coordinates).

fMR Imaging Results. Significant BOLD signal increase was observed extensively throughout the brain for each subject (Table 2), including in the lamina terminalis (two subjects), anterior cingulate

Table 1. The PET activations and deactivations relative to the baseline scan (rest) as determined after 25 min of hypertonic saline infusion when thirst sensation was beginning

Region*	BA	Talairach coordinates,† mm			Cluster size, voxels	*Z* score	*P* value
		x	*y*	*z*			
Activations							
L. culmen		−28	−44	−20	60	4.04	<0.00003
L. parahippocampal gy.	28	−19	−26	−10	67	3.94	0.00004
R. middle occipital gy.	37	38	−64	8	40	3.81	0.00007
L. posterior cingulate	29	−6	−44	6	43	3.72	0.00010
R. thalamus		19	−22	2	51	3.72	0.00010
R. cuneus	18	4	−88	22	47	3.69	0.00011
L. insula	13	−37	−22	18	68	3.66	0.00013
L. cerebellar lingual		−2	−40	−8	53	3.66	0.00013
R. declive		18	−82	−22	77	3.66	0.00013
R. precuneus	7	6	−54	32	48	3.63	0.00014
L. fusiform gy.	37	−30	−48	−10	48	3.59	0.00016
R. parahippocampal gy.	30	14	−42	−4	55	3.56	0.00018
L. cingulate gy.	24	−9	−4	36	44	3.53	0.00021
R. inferior frontal gy.	47	28	22	−16	38	3.53	0.00021
L. declive		−36	−54	−16	63	3.53	0.00021
L. culmen		−42	−45	−32	39	3.53	0.00021
Deactivations							
R. nodule		6	−54	−28	56	−4.18	<0.00003
R. caudate tail		22	−38	12	62	−4.11	<0.00003
R. midbrain/pons		4	−8	−18	61	−4.02	<0.00003
R. caudate		12	2	18	59	−3.99	0.00003
L. cerebellar tonsil		−1	−52	−36	61	−3.95	0.00004
L. caudate		−2	8	6	72	−3.92	0.00004
L. caudate		−20	−18	22	42	−3.89	0.00005
L. caudate head		−8	10	0	68	−3.86	0.00006
L. fusiform gy.	37	−44	−44	−10	65	−3.83	0.00006
R. cingulate gy.	31	1	−42	28	78	−3.76	0.00008
L. medial frontal gy.	6/8	−6	18	44	62	−3.73	0.00009
R. medial frontal gy.	6/8	4	32	38	61	−3.70	0.00011
R. precentral gy.		32	2	32	48	−3.70	0.00011
R. subcallosal gy.	25	12	10	−16	69	−3.70	0.00011
R. anterior cingulate		21	34	6	62	−3.64	0.00014
R. anterior cingulate		18	30	−6	65	−3.64	0.00014

For activation, Z score > 3.52 ($P < 0.0005$) and cluster size > 25 voxels.
*L., left; R., right; Gy., gyrus.
†Brain atlas coordinates are in millimeters along left–right (*x*), anterior–posterior (*y*), and superior–inferior (*z*) axes.

in all subjects (BA 24/32), and mid-cingulate (BA 24/31) in two subjects (Fig. 3). Three of the four subjects had bilateral insula activations (BA 13). One subject showed significant activation in the left parahippocampal gyrus (BA 35), and two subjects showed activation in the frontal lobes, including the precentral gyrus bilaterally (BA 4/6), right middle frontal gyrus (BA 9), and left superior frontal gyrus (BA 10). In three subjects, regions of activity were also observed in the angular gyrus (BA 39), bilaterally, and the parietal lobe, including the right superior parietal lobule (BA 7) and postcentral gyrus (BA 1/2/3).

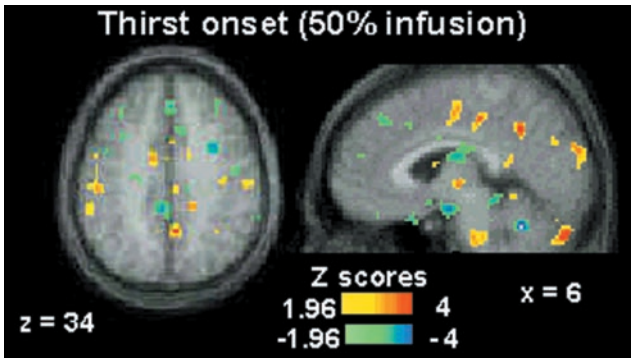

Fig. 2. The brain activations shown on the average magnetic resonance anatomical image of the 10 subjects in the PET experimental cohort, in which the scans from the baseline nonthirsty state were compared with the scans acquired after 50% of the hypertonic saline infusion was administered (25 min after the commencement of the infusion). (*Left*) Activation in the post-cingulate gyrus ($Z = 34$). (*Right*) Mid-cingulate, precuneus, and cerebellar activations.

Activation was also observed in a number of other regions (data not shown), particularly for subject 2. Subject 1 showed additional activation in the left uncus [Talairach coordinate (−24, 3, −21); Z score = 6.18]. For subjects 2 and 3, activation was observed in the visual cortex in the right occipital gyrus [(33, −87, 0); Z score = 3.90], left middle occipital gyrus [(−21, −90, 12); Z score = 6.1], and left cuneus [(−3, −75, 9); Z score = 3.54]. Significant activations were also observed for subject 2 in the left caudate [(−6, 6, 6); Z score = 5.61] and brainstem in the vicinity of the red nucleus [(0, −18, −3); Z score = 3.34]. Significant activations were observed for subject 3 in the right caudate [(12, −6, 18); Z score = 5.05] and left mamillary body [(−6, −9, −15); Z score = 5.93]. Subject 4 showed significant activation in the left lobe of the cerebellum in the tonsil [(−30, −45, −42); Z score = 7.34], pyramis [(−9, −75, −30); Z score = 6.67], and anterior lobe [(18, −42, −30); Z score = 5.27].

Discussion

The lamina terminalis is a crucial site in the brain for osmotically stimulated water-drinking in animals. Ablation of this region causes permanent or temporary adipsia (20), and c-*fos* expres-

Table 2. The fMR activations for each subject during their 10-min period of maximum thirst score increase, with *Z* score > 3.54 (*P* < 0.005) with cluster size >15 voxels

		Subject 1					Subject 2					Subject 3					Subject 4				
Region of activation	BA	*x*	*y*	*z*	Cl. size	*Z* score	*x*	*y*	*z*	Cl. size	*Z* score	*x*	*y*	*z*	Cl. size	*Z* score	*x*	*y*	*z*	Cl. size	*Z* score
Limbic																					
R. ant. cingulate†	24/25/32	6	42	−21	473	>6.48	0	12	−3	152	5.72										
R. ant. cingulate	24/25/32																12	42	−9	91	6.75
L. ant. cingulate	32						−3	45	0	33	3.65*	−12	39	−3	92	4.06					
L. cingulate gy.	31						−3	−75	9	26	3.54*										
L. insula	13											−39	24	3	15	4.46	−39	12	12	301	>6.6
R. insula	13	39	15	12	130	4.85	51	−9	6	21	3.56*										
L. parahippocampal gy.	36											−33	−51	−24	62	5.7					
R. parahippocampal gy.	27																24	−33	0	30	5.24
Frontal lobe																					
R. precentral gy.	6						63	−3	36	23	4.06*										
L. precentral gy.	4	−36	−24	60	257	>7.51	−57	−6	21	53	5.45										
	6	−30	−12	63	33	5.67	−24	3	51	40	4.69										
R. inf. frontal gy.	45/47																36	42	0	43	>7.8
																	60	18	18	163	7.57
L. inf. frontal gy.	44						−63	15	9	171	6.64										
R. middle frontal gy.	6/9/46	30	39	36	82	6.98	39	18	27	54	3.12*										
		60	18	30	130	6.88	39	15	57	54	4.5*										
		45	51	15	88	6.49															
L. middle frontal gy.	6/8/9						−51	15	27	171	4.94						−30	9	54	89	5.39
L. sup. frontal gy.	9/10/11	6	42	−21	473	>7.51	−9	45	33	49	5.29	−21	48	0	92	5.94					
							−9	48	−12	58	5.04										
R. medial frontal gy.	25						3	27	−15	250	5.05*										
L. medial frontal gy.	6/10	0	−6	48	93	6.84	−12	57	0	33	3.67*										
Parietal lobe																					
R. postcentral gy.	1	66	−18	33	52	5.69	51	−24	54	39	4.76										
L. postcentral gy.	3/5						−48	−18	45	36	4.86	−30	−42	66	51	6.16					
R. sup. parietal lobule	7	−24	−48	57	53	7.39	36	−69	51	77	3.98										
R. precuneus	7	18	−81	36	351	7.24															
R. angular gy.	39						57	−60	33	77	5.41										
L. angular gy.	39						−45	−69	36	32	5.03										
Temporal lobe																					
R. sup. temporal gy.	22	60	6	0	78	7.23	15	27	60	15	3.42*						69	−24	3	67	6.83
L. sup. temporal gy.	22	−57	15	−3	174	7.51											−57	0	3	301	>7.8
R. middle temporal gy.	21																66	−30	−6	30	5.65
L. middle temporal gy.	21						−51	−27	−6	19	3.28*										

Data for each subject were recorded during the 10-min period in which the rate of thirst score increase was maximal: 15–25 min, 20–30 min, 2.5–12.5 min, and 12.5–22.5 min for subjects 1, 2, 3, and 4, respectively. However, *Z* score values for subject 2 that are marked with an asterisk were taken from the 7.5- to 17.5-min period. The coordinates for each cluster indicate the position of the peak voxel in Talairach space, with the anatomical labels determined by visual inspection of each subject's activations rendered on their normalized anatomy. Cl., cluster; R., right; L., left; gy., gyrus, ant., anterior; sup., superior; inf., inferior.
†Includes the organ vasculosum of the lamina terminalis.

sion occurred in this region with dipsogenic stimuli (21). Tumors in this region in humans also cause disruption of thirst and hypernatraemia (22).

PET scans taken 25 min after beginning the hypertonic infusion show that at this early stage there was significant activation in the parahippocampus and cingulate gyrus bilaterally and in the left insula. Activation occurred in the left posterior cingulate gyrus and in the precuneus and cuneus regions, which have strong connections with the posterior cingulate. There was activation in the ventral postcentral gyrus, probably associated with commencement of a "dry mouth" sensation. Extensive cerebellar activations occurred in the culmen, declive, pyramis, and lingual; as discussed elsewhere (23), these may reflect sensory processing rather than, or as well as, intention of motor behavior directed toward drinking. Some areas activated in maximum thirst, including the middle and transverse temporal and parietal areas, were not activated at this early stage, and this pattern of activation may reflect cognitive processes taking place after thirst was well established. It was notable that there was no activation ($P < 0.001$) in the BA 32 area, which was immediately anterior to the genu of the corpus callosum. However, there was major activation in BA 32 in the fMR activation maps when there was a rapid increase in thirst score. Indeed, the anterior cingulate areas identified by using fMR were very close to the principal cingulate area emerging in the PET study of maximum thirst, strengthening the possibility that this area is involved with the consciousness of thirst. Our observations are consistent with the fact that large stimulus-bound drinking occurs with electrical stimulation in that region in conscious monkeys (12).

Overall, at the earliest stage of the sensation of thirst, many phylogenetically ancient regions of the brain were activated, as was found when thirst sensation was maximal. Particular regions that were activated, which can be noted in both circumstances, were the anterior and posterior cingulate, parahippocampal gyrus, insula, inferior frontal gyrus, and cerebellum. There was also a strong deactivation in the rostral ventral pons, which was also conspicuous during maximum thirst. The role of the anterior cingulate in states involving primal emotion has emerged from several studies (3, 12). The area has wide connections with systems involved in vegetative behaviors. In particular, this region is the part of the cortex with the most extensive connections to the hypothalamus.

The fMR Imaging of Thirst. In this study, the advantages of fMR over PET relate to the shorter whole-brain scanning duration (3 s) and the ability to scan continuously throughout the experimental procedure (excluding the drinking period). These advantages enabled the identification of brain activity occurring at the time of the

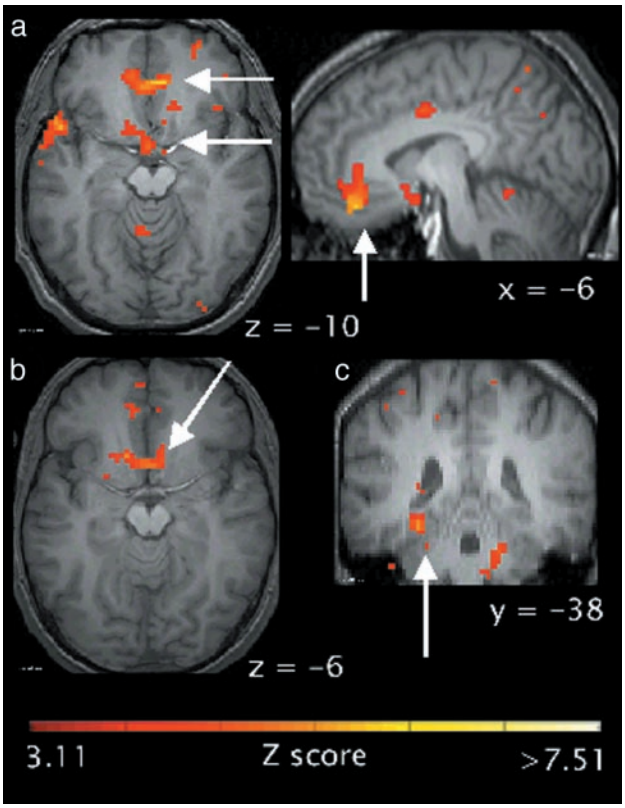

Fig. 3. An fMR representative imaging section for three subjects highlighting (arrows) areas of significant BOLD signal increase during the 10-min period of highest increase of thirst score. (*a*) Anterior cingulate cortex and hypothalamus for subject 1. (*b*) Lamina terminalis for subject 2. (*c*) Left parahippocampal gyrus for subject 4.

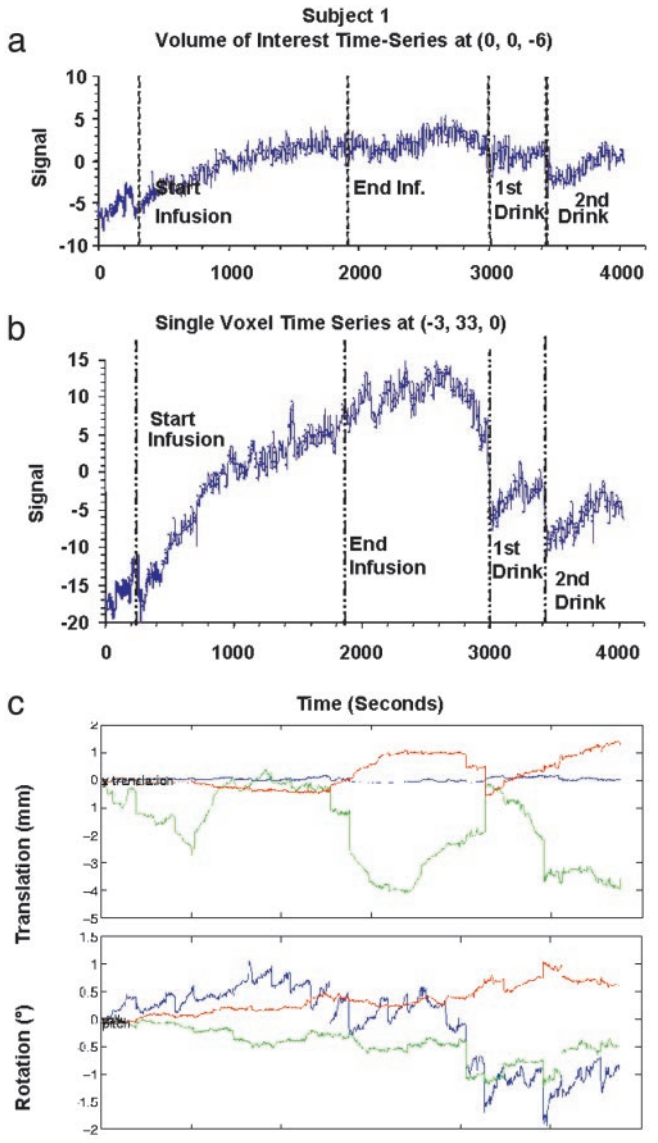

Fig. 4. The BOLD signal time scores response for the lamina terminalis (*a*) and anterior cingulate (*b*) voxels of interest at Talairach coordinates (0, 0, −6) and (−3, 33, 0), respectively, for subject 1. (*c*) Translational motion (*x*, *y*, *z* directions shown in blue, green, and red, respectively) and rotational motion (pitch, roll, and yaw shown in blue, green, and red, respectively) correction parameters as determined from SPM. Note that the period during which the largest head motion occurred did not correlate with the rapid change of BOLD signal in BA 32.

greatest change of thirst score. The period of rapid increase of thirst score provided activation maps within a 10-min period, whereas our preceding PET studies could acquire only two PET scans during this period.

The disadvantages of fMR relative to PET in an experiment of this type mainly reside in extended-period (in excess of ≈10 min) EPI signal instability caused by machine and physiological effects (24, 25). This limitation precluded correlation of the thirst score changes with the full time series data set, as we previously examined in our PET studies. Nevertheless, identification of regions of activation based on the 10-min period of maximum increase of thirst score directed subsequent examination of the full time series of *a priori* hypothesized regions of interest (lamina terminalis, anterior cingulate, and parahippocampal gyrus), revealing fascinating changes in these areas.

In two of the four subjects there was strong activation in the region of the anterior wall of the third ventricle, which includes the organ vasculosum of the lamina terminalis. The animal data on the involvement of this region in thirst are noted in the Introduction. The fMR showed powerful activation in the principal telencephalic regions, which were shown by PET. There was strong activation of the insula in all subjects and in the cingulate gyrus (either BA 32 or BA 24 in all subjects). Activation also occurred in the left parahippocampal gyrus in one subject and in the region of the frontal lobes in all subjects. In three subjects, the postcentral gyrus of the parietal lobe was activated bilaterally, probably related to the dry mouth sensation. Across the group, several other regions were activated, including the cerebellum, cuneus, angular gyrus, brainstem (red nucleus region), and mammillary bodies in the hypothalamus. Despite some variability, the fMR data were nevertheless consistent with the PET studies in terms of the telencephalic areas activated, and it crucially delineated the lamina terminalis involvement in thirst in human studies, as has been demonstrated experimentally in animal studies.

We have been able to make robust observations of continual BOLD signal increase throughout the period of hypertonic saline infusion. Furthermore, the BOLD signal stability itself was unlikely to have been influenced by an osmolality change, given the dramatic change of signal after drinking, when no osmolality change occurred. Of particular importance is that periods of greatest head movements (<3 mm) do not correlate with the times at which major signal changes occur. The practical difficulties of drinking while lying prone in the magnetic resonance scanner and the consequence that small head motion may occur during the process of drinking were examined carefully. By using well validated algorithms (26), a small amount of translational and rotational head motion was

PHYSIOLOGY

observed but corrected for by using a rigid body transformation. The lamina terminalis volume of interest (7 voxels) time series demonstrates steady fMR signal increases during the infusion period but relative stability in the immediate period after drinking. This signal profile from the anterior wall of the third ventricle does not show changes related to head movement, except perhaps during the second drink taken by subject 1. The period during which the largest head motion occurred did not correlate with the rapid change of BOLD signal in BA 32 or with the precipitative signal decline in that region after drinking. This finding is demonstrated in Fig. 4*b*, where a single voxel in the anterior cingulate shows constant signal increase throughout the infusion and precipitate decline after drinking. A similar effect of drinking was seen in these two areas in subject 2. Although these preliminary observations should be considered with caution in the light of technical limitations, they suggest the need for further experiments, including one using perfusion fMR.

A particular possibility of a productive direction of future research relates to a general issue. Crick (27) has stated that it seems probable "that at any one moment some active neural processes in your head correlate with consciousness while others do not. What are the differences between them?" Discussing vision, he adds that it seems the brain has to impose some global unity on certain activities in its different parts so the attributes of a single object (e.g., its shape, color, movement, location, and so on) are in some way brought together without at the same time confusing them with the attributes of other objects in the visual field. Further, this global process requires mechanisms that could well be described as attention or as perceptual binding, and such global unity might be expressed by the correlated firing of the neurons involved. It is highly probable that not all neurons that are active are supporting conscious perception at the time they are active. Edelman and Tononi (28) suggest that at any given time only a subset of neuronal groups in the human brain (although not a small subset) directly contribute to conscious experience, raising the question as to which group at any particular time is special and the question of how these groups should be identified. They postulate that there is a cluster of neuronal groups they call a "dynamic core" that strongly interact among themselves but have distinct functional borders with the rest of the brain. The core has both an integrated and constantly changing composition, as the term "dynamic" suggests. This distributed functional cluster achieves high integration through reentrant interactions in the thalamocortical system. From neuroimaging studies, it could be postulated that the dynamic core common to several primary emotions would include regions in the anterior and posterior cingulate gyri, insula, parahippocampal gyri, orbitofrontal gyri, and thalamus.

With regard to these statements by Crick and Edelman and Tononi, it is cogent that the biological organization of thirst and other specific appetites, like salt appetite, involves a capacity for very rapid satiation. The depleted animal will drink very rapidly to satiation, after which there is a precipitate decline in interest, and then thirst disappears. This rapid satiation has very high survival advantage for animals in so far as they can correct a deficit quickly and then exit a place, like a water hole, where they will be particularly vulnerable to predators who may lie in wait there.

The fMR results showed a most interesting comparison between responses in the anterior cingulate region, which disappeared immediately after satiation, and the activation in the anterior wall of the third ventricle, which persisted with drinking. The continued BOLD signal increase in the lamina terminalis is consistent with the fact that the raised Na concentration persisted and would only change gradually with progressive absorption of water into the bloodstream. Thus, putatively, the activated state in this area could be attributed directly to the increased plasma Na concentration. Conversely, the activation in the anterior cingulate could be subserving the consciousness of thirst, decreasing precipitously by the loss of consciousness of thirst consequent on the act of drinking water to satiation.

The observation warrants further experimental study, with the prospect of a delineation of those regions subserving consciousness of thirst from those responding at a nonconscious level to chemical change in the body. In a sense, this type of experiment addresses Crick's question, and the experimental circumstance where change of conscious state is contrived by change of the chemical milieu of the body may be an apt paradigm insofar as it deals with basic vegetative systems of high survival value.

It can be foreseen that analysis of the phenomenon of thirst, including genesis, consciousness, and satiation, will be more complex than indicated above. It is well attested that water-drinking in dehydrated animals of different species can largely inhibit antidiuretic hormone (ADH) secretion in 3–10 min (29–31). This inhibition occurs long before the consumed water could reduce the high plasma Na concentration and osmotic pressure that initiated the secretion of ADH. Thus, it may be that gratification may "turn off" some areas that specifically subserve the consciousness of thirst and others that control neuroendocrine regulation of ADH secretion, which may be at a nonconscious level, and are topographically different from those controlling consciousness.

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1. Denton, D., Shade, R., Zamarripa, F., Egan, G., Blair-West, J., McKinley, M., Lancaster, J. & Fox, P. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 5304–5309.
2. Robinson, B. W. & Mishkin, M. (1968) *Exp. Brain Res.* **4,** 330–366.
3. Liotti, M., Brannan, S., Egan, G., Shade, R., Madden, L., Abplanalp, B., Robillard, R., Lancaster, J., Zamarripa, F., Fox, P. T. & Denton, D. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 2035–2040.
4. Corfield, D. R., Fink, G. R., Ramsay, S. C., Murphy, K., Harty, H. R., Watson, J. D. G., Adams, L., Frackowiak, R. S. J. & Guz, A. (1995) *J. Physiol (London)* **48,** 77–84.
5. Banzett, R. B., Mulnier, H. E., Murphy, K., Rosen, S. D., Wise, R. J. S. & Adams, L. (2000) *NeuroReport* **11,** 2117–2120.
6. Tataranni, P. A., Gautier, J.-F., Chen, K., Uecker, A., Bandy, D., Salbe, A. D., Pratley, R. E., Lawson, M., Reiman, E. M. & Ravussin, E. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 4569–4574.
7. Blok, B. F. M., Willemsen, A. T. M. & Holstege, G. (1997) *Brain* **129,** 111–121.
8. Noto, H., Ropplo, J. R., Steers, W. D. & de Groat, W. C. (1991) *Brain Res.* **549,** 95–105.
9. Blok, B. F., de Weerd, H. & Holstege, G. (1995) *J. Comp. Neurol.* **359,** 300–309.
10. Rainville, P., Duncan, G. H., Price, D. D., Carrier, B. & Bushnell, M. C. (1997) *Nature* **277,** 968–971.
11. Coghill, R. C., Talbot, J. D., Evans, A. C., Meyer, E., Gjedde, A., Bushnell, M. C. & Duncan, G. H. (1994) *J. Neurosci.* **14,** 4095–4108.
12. Sewards, T. V. & Sewards, M. A. (2003) *Brain Res. Bull.* **61,** 25–49.
13. Denton, D. A., McKinley, M. J. & Weisinger, R. S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 7397–7404.
14. Fox, P. T., Perlmutter, J. S. & Raichle, M. E. (1985) *J. Comput. Asst. Tomogr.* **9,** 141–153.
15. Fox, P. T. & Mintun, M. A. (1988) *J. Nucl. Med.* **30,** 141–149.
16. Denton, D., Shade, R., Zamarripa, F., Egan, G., Blair-West, J., McKinley, M. & Fox, P. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 2532–2537.
17. Talairach, J. & Tournoux, P. (1988) *Coplanar Stereotaxic Atlas of the Human Brain* (Thieme, New York).
18. Lancaster, J. L., Glass, T. G., Lakipalli, B. R., Downs, H., Mayberg, H. & Fox, P. T. (1995) *Hum. Brain Mapp.* **3,** 209–223.
19. Friston, K. J., Holmes, A. P., Price, C. J., Buchel, C. & Worsley, K. J. (1999) *NeuroImage* **10,** 385–396.
20. Andersson, B., Leksell, L. & Lishajko, F. (1975) *Brain Res.* **99,** 262–275.
21. McKinley, M. J., Hards, D. K. & Oldfield, B. J. (1994) *Brain Res.* **653,** 305–314.
22. Robertson, G. L. (1991) in *Thirst: Physiological and Psychological Aspects*, eds. Ramsay, D. J. & Booth, D. (Spring, London), pp. 453–477.
23. Parsons, L. M., Denton, D., Egan, G., McKinley, M., Shade, R., Lancaster, J. & Fox, P. T. (2000) *Proc. Nat. Acad. Sci. USA* **97,** 2332–2336.
24. Weisskoff, R. M. (1996) *Magn. Reson. Med.* **36,** 643–645.
25. Kruger, G. & Glover, G. H. (2001) *Magn. Reson. Med.* **46,** 631–637.
26. Friston, K. J., Holmes, A. P., Worsley, K. J., Poline, J.-P., Frith, C. D. & Frackowiak, R. S. J. (1995) *Hum. Brain Mapp.* **2,** 189–210.
27. Crick, F. (1994) *The Astonishing Hypothesis: The Scientific Search for the Soul* (Scribner, New York).
28. Edelman, G. M. & Tononi, G. (2000) *A Universe of Consciousness: How Matter Becomes Imagination* (Basic Books, New York).
29. Rougon Papuzzi, G., Cau, P., Boudier, J. A. & Cupo, A. (1978) *Neuroendocrinology* **27,** 46–62.
30. Vincent, J. D., Arnauld, E. & Broulac, B. (1972) *Brain Res.* **44,** 371–384.
31. Nicolaides, S. (1969) *Ann. N.Y. Acad. Sci.* **157,** 1176–1203.